

## **Supplemental Information**

### **Telomeric overhang length determines structural dynamics and accessibility to telomerase and ALT associated proteins**

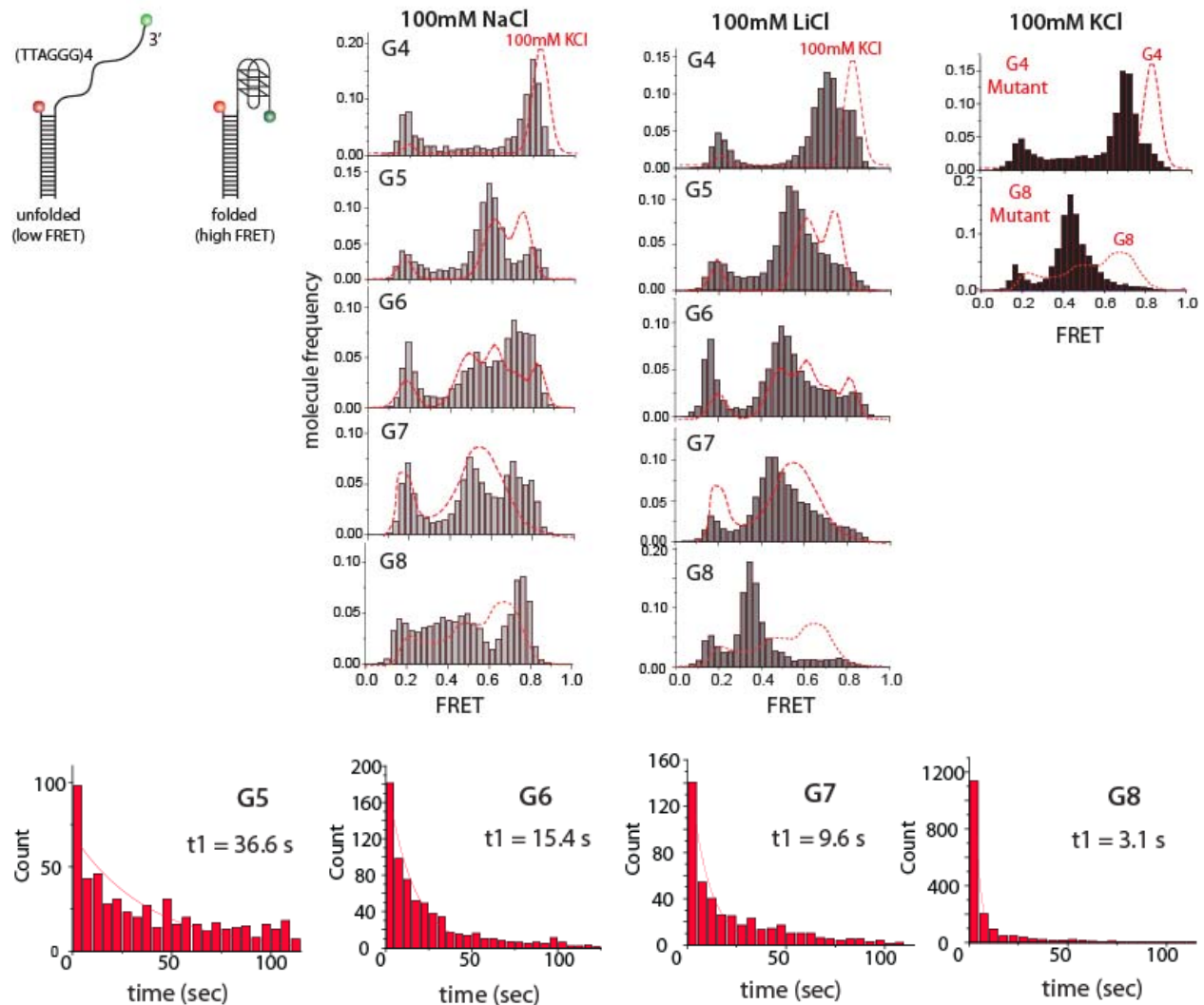
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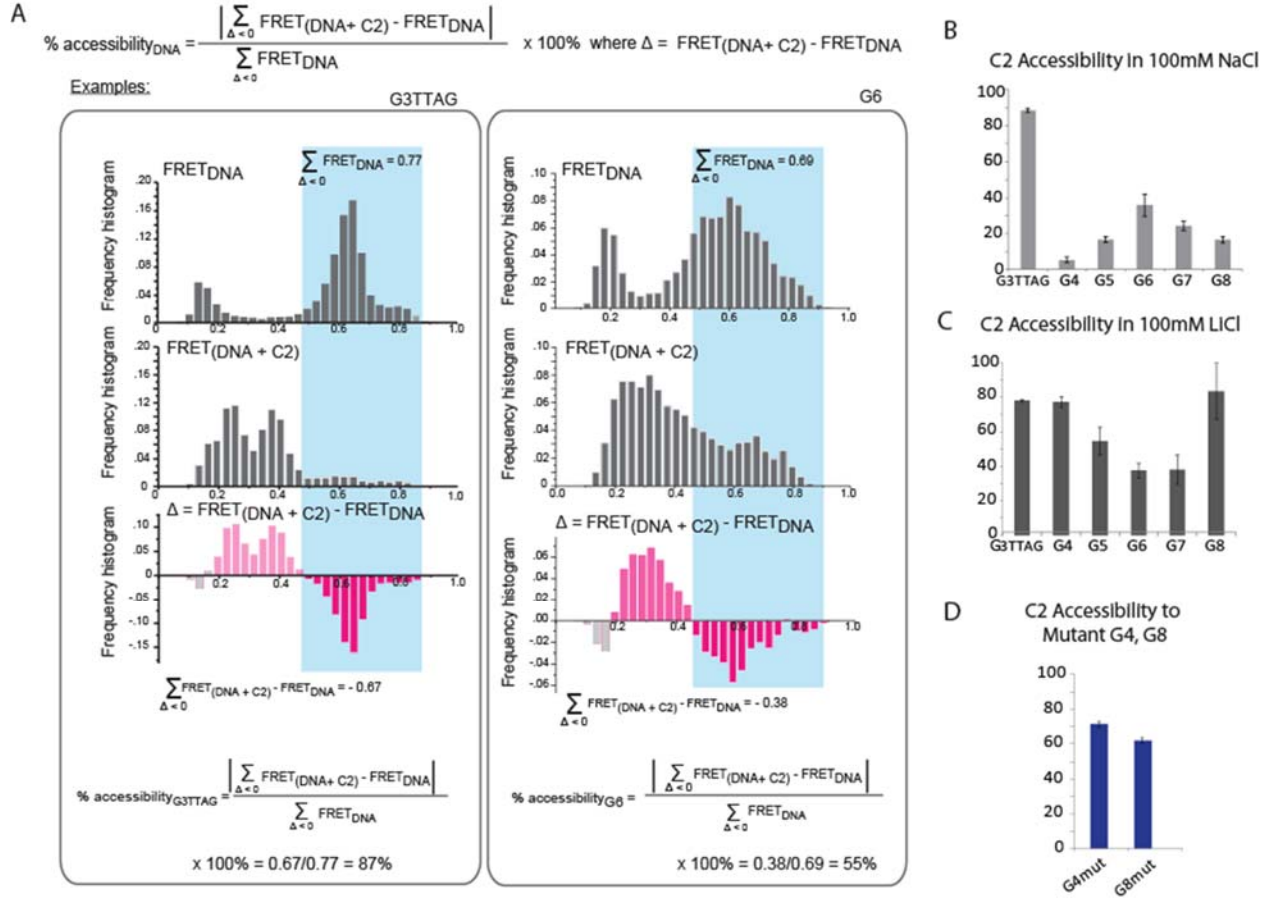
Figures S1-3

## Supplemental Figure S1, related to Figure 1



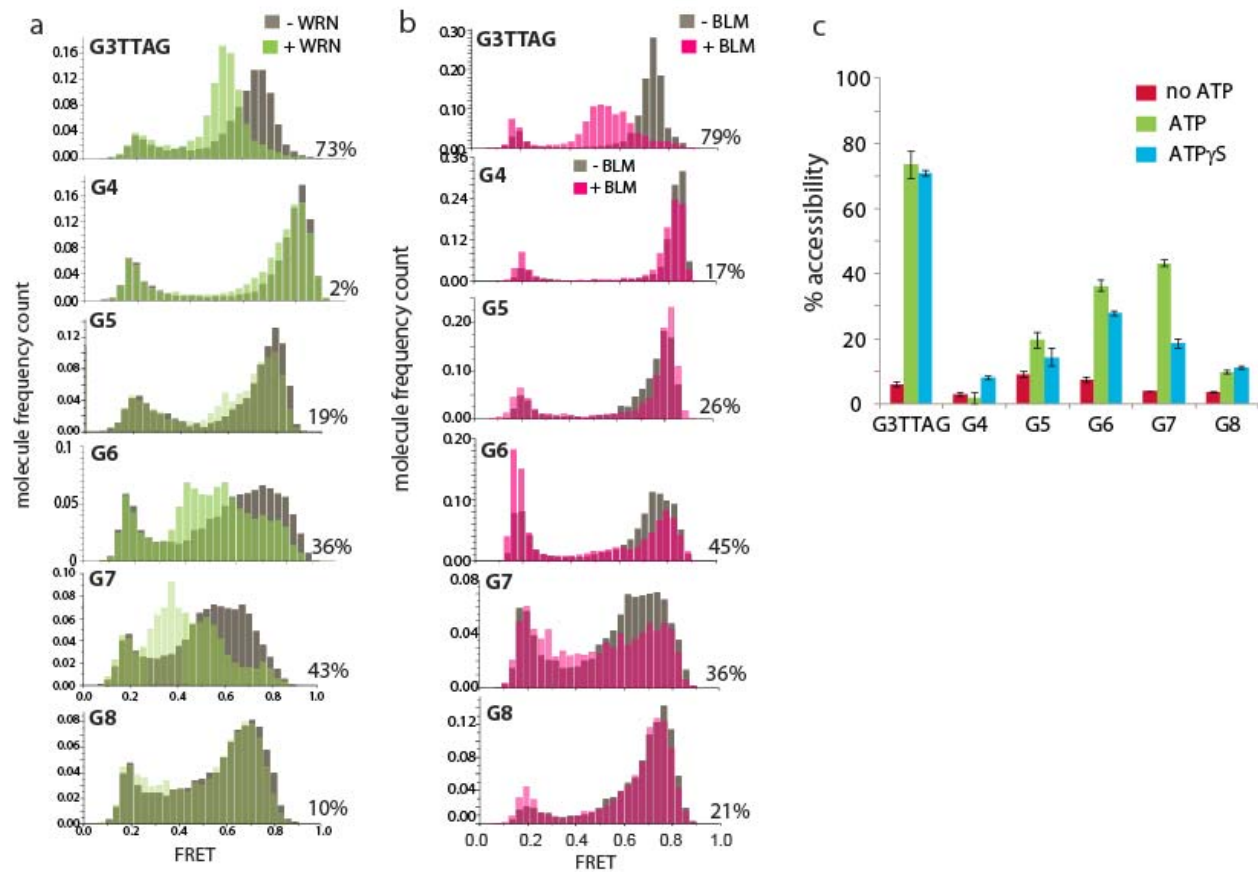
**Figure S1** G4-G8 DNA folding was monitored in 100mM NaCl and 100mM LiCl. In all histogram analysis, the red dotted line is the outline of the KCl histograms superposed to the NaCl and LiCl data for comparison. The histogram analysis shows that in NaCl, the folding of G4-G8 resemble the folding states seen in KCl i.e high FRET peak for G4, two peaks for G5 and presence of high FRET in G8. In contrast, LiCl induced a broad FRET distribution for all lengths. The lack of high FRET peak for G8, together, in particular reflects lack of GQ folding induced in LiCl condition. The G4 and G8 mutant sequence which had the terminal (4<sup>th</sup> and 8<sup>th</sup>) repeat changed to TTAGC<sub>G</sub> showed lack of tight folding in both cases. These measurements show that the G4-G8 folding observed in KCl arises from potassium induced dynamic folding specific to telomeric overhang sequence. (BOTTOM) Dwell time distribution of G5-G8.

## Supplemental Figure S2, related to Figure 2



**Figure S2** (A) FRET histograms are plotted for DNA-only ( $\text{FRET}_{\text{DNA}}$ ) and after addition of C2 ( $\text{FRET}_{\text{DNA}+\text{C2}}$ ). The  $\Delta$ (delta FRET) is calculated by subtracting the  $\text{FRET}_{\text{DNA}}$  histogram from the  $\text{FRET}_{\text{DNA}+\text{C2}}$  histogram. The percent accessibility is calculated by summing the portion with the negative FRET change (dark fuschia) and dividing by the sum of the relative frequencies of the starting histograms within the blue shaded area. (B) C2 accessibility tested for G4-G8 in 100mM NaCl shows a similar pattern as seen in KCl condition i.e limited accessibility to G4 and G8 and high accessibility to G5-G7. (C) C2 accessibility tested for G4-G8 in 100mM LiCl shows that the accessibility is high for all DNAs. We note that the accessibility for G5-G7 are underestimated due to the broad histograms before and after C2 addition, that overlap significantly. (D) C2 accessibility tested for G4 and G8 mutants both show high accessibility, indicating the unfolded conformation that lead to high occupancy by C2.

**Supplemental Figure S3**, related to Figure 3



**Figure S3** (A) FRET histograms taken for G4-G8 in the presence of WRN helicase. (B) FRET histograms taken for G4-G8 in the presence of BLM helicase. They both exhibit limited binding to G4 and G8 and high accessibility for G3TTAG and G5-G7. (C) Accessibility of WRN measured in the absence of ATP and in the presence of ATP $\gamma$ S.